

5/PRTS

10/540302
JC09 Rec'd PCT/PTO 21 JUN 2005

COURTESY COPY OF AMENDMENTS ACCORDING TO ARTICLE 34 OF PCT

Claims

1. (Amended) A method for inducing somatic cell homologous recombination, characterized by being a method for inducing somatic cell homologous recombination in eukaryotic organism cells wherein DNA homologous recombination is occurring at an arbitrary genetic locus, and wherein, by controlling the transcription of said gene by placing a transcription promoter for the transcription of a gene at said genetic locus on the downstream 3' side of a base sequence similar to the base sequence of said gene, being action-capably adjacent to said gene, DNA homologous recombination between the base sequence of said gene and a base sequence similar to said gene is induced.
2. A method recited in Claim 1, characterized in that the aforementioned cells are DT40 cells.
3. (Deleted)
4. A method recited in any of Claims 1 through 3, characterized in that a cis-acting region for the aforementioned transcription control contains either one or both of an enhancer and a nuclear matrix attachment region (MAR).
5. A method recited in any of Claims 1 through 4, characterized in that, when the aforementioned gene and a base sequence similar to the aforementioned gene are exogenous, the following steps are included:
 - (a) a step wherein the order on a vector of the base sequence similar to said gene, a transcription promoter, and said gene, are, from the 5' side, in the order: base sequence similar to said gene, transcription promoter, said gene; and said transcription promoter is inserted in a manner so as to be capable of acting on said gene;
 - (b) a step wherein said vector is introduced into a cell, and a base sequence similar to said gene, a transcription promoter, and said gene is incorporated onto a chromosome.
6. A method recited in Claim 5, characterized in that either one or both of an enhancer and a nuclear matrix attachment region (MAR) are inserted onto the aforementioned vector in a manner so as to be capable of acting on the aforementioned transcription promoter.

7. A method recited in Claim 5 or 6, characterized in that the aforementioned transcription promoter is an inducible promoter.
8. A method recited in Claim 7, characterized in that the aforementioned inducible promoter is a tetracycline inducible promoter.
9. A method recited in any one of Claims 5 through 8, characterized in that the aforementioned gene is an enhanced cyan fluorescent protein (ECFP) gene.
10. A method recited in any one of Claims 5 through 9, characterized in that the base sequence similar to the aforementioned gene is an enhanced green fluorescent protein (EGFP) genetic sequence.
11. A method recited in any one of Claims 4 through 10, characterized in that the aforementioned enhancer is a chicken antibody light chain gene enhancer (3' enhancer), and the aforementioned nuclear matrix attachment region (MAR) is chicken derived.
12. A cell wherein DNA homologous recombination is induced by a method recited in any one of Claims 1 through 11.
13. A gene for which homologous recombination is induced by a method recited in any one of Claims 1 through 11.
14. A protein encoded by a gene for which homologous recombination is induced recited in Claim 13.
15. A vector wherein a gene that induces homologous recombination and a transcription promoter for controlling transcription of said gene are placed, a base sequence similar to said gene is placed in a region upstream on the 5' side of said transcription promoter, and constructed in order to induce homologous recombination of said gene.
16. A vector recited in Claim 15, wherein one or both of an enhancer and a nuclear matrix attachment region (MAR) are further inserted in a manner so as to be capable of acting.

Claims

1. A method for inducing somatic cell homologous recombination, characterized by being a method for inducing somatic cell homologous recombination in eukaryotic organism cells wherein DNA homologous recombination is occurring at an arbitrary genetic locus, and wherein, by controlling transcription of a gene at said genetic locus, DNA homologous recombination between the base sequence of said gene and a base sequence similar to said gene is induced.
2. A method recited in Claim 1, characterized in that the aforementioned cells are DT40 cells.
3. A method recited in Claim 1 or 2, characterized in that a transcription promoter for the aforementioned transcription control is located downstream on the 3' side of a base sequence similar to the aforementioned gene, and by being action-capably adjacent to the aforementioned gene, controls the transcription of said gene.
4. A method recited in any of Claims 1 through 3, characterized in that a cis-acting region for the aforementioned transcription control contains either one or both of an enhancer and a nuclear matrix attachment region (MAR).
5. A method recited in any of Claims 1 through 4, characterized in that, when the aforementioned gene and a base sequence similar to the aforementioned gene are exogenous, the following steps are included:
 - (a) a step wherein the order on a vector of the base sequence similar to said gene, a transcription promoter, and said gene, are, from the 5' side, in the order: base sequence similar to said gene, transcription promoter, said gene; and said transcription promoter is inserted in a manner so as to be capable of acting on said gene;
 - (b) a step wherein said vector is introduced into a cell, and a base sequence similar to said gene, a transcription promoter, and said gene is incorporated onto a chromosome.
6. A method recited in Claim 5, characterized in that either one or both of an enhancer and a nuclear matrix attachment region (MAR) are inserted onto the aforementioned vector in a manner so as to be capable of acting on the aforementioned transcription promoter.
7. A method recited in Claim 5 or 6, characterized in that the aforementioned transcription promoter is an inducible promoter.

8. A method recited in Claim 7, characterized in that the aforementioned inducible promoter is a tetracycline inducible promoter.
9. A method recited in any one of Claims 5 through 8, characterized in that the aforementioned gene is an enhanced cyan fluorescent protein (ECFP) gene.
10. A method recited in any one of Claims 5 through 9, characterized in that the base sequence similar to the aforementioned gene is an enhanced green fluorescent protein (EGFP) genetic sequence.
11. A method recited in any one of Claims 4 through 10, characterized in that the aforementioned enhancer is a chicken antibody light chain gene enhancer (3' enhancer), and the aforementioned nuclear matrix attachment region (MAR) is chicken derived.
12. A cell wherein DNA homologous recombination is induced by a method recited in any one of Claims 1 through 11.
13. A gene for which homologous recombination is induced by a method recited in any one of Claims 1 through 11.
14. A protein encoded by a gene for which homologous recombination is induced recited in Claim 13.
15. A vector wherein a gene that induces homologous recombination and a transcription promoter for controlling transcription of said gene are placed, a base sequence similar to said gene is placed in a region upstream on the 5' side of said transcription promoter, and constructed in order to induce homologous recombination of said gene.
16. A vector recited in Claim 15, wherein one or both of an enhancer and a nuclear matrix attachment region (MAR) are further inserted in a manner so as to be capable of acting.